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Fertile *Prototaxites taiti*: a basal ascomycete with inoperculate, polysporous asci lacking croziers

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Running heads: *Prototaxites*, a basal ascomycete

Authors' contributions

~~RH recognised the fertile parts, did the light and most of the scanning electron microscopy, wrote most of the manuscript; DE~~ RH did the light and most of the scanning electron microscopy and the mycological interpretation, wrote most of the manuscript; DE was initially investigating specimens of Prototaxites with intact surface layer from various slide collections, wrote part of the manuscript, provided the material and infrastructure, organized financial support and helped draft the manuscript. LA prepared the specimens for SEM analysis, helped with LM and SEM investigations and contributed to the the manuscript. CS-D did the

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confocal microscopy and contributed to the manuscript. All authors gave final approval for publication.

The affinities of *Prototaxites* have been debated ever since its fossils, some attaining tree-trunk proportions, were discovered in Canadian Lower Devonian rocks in 1859. Putative assignations include conifers, red and brown algae, liverworts and fungi (some lichenised). Detailed anatomical investigation led to the reconstruction of the type species, *P. logani*, as a giant ~~fruiting body~~ sporophore (basidioma) of an a~~A~~garicomycete (= ~~H~~holobasidiomycete), but evidence for its reproduction remained elusive. Tissues associated with *P. taiti* in the Rhynie chert plus charcoalified fragments from southern Britain are investigated here to describe the reproductive characters and hence affinities of *Prototaxites*. Thin sections and peels (Pragian Rhynie chert, Aberdeenshire) were examined using light and confocal microscopy; Přídolí and Lochkovian charcoalified samples (Welsh Borderland) were liberated from the rock and examined with scanning electron microscopy. *Prototaxites taiti* possessed a superficial hymenium comprising an epihymenial layer, delicate septate paraphyses, inoperculate polysporic asci lacking croziers and a subhymenial layer composed predominantly of thin-walled hyphae and occasional larger hyphae. *Prototaxites taiti* combines features of extant Taphrinomycotina (Neolectomycetes lacking croziers) and Pezizomycotina (epihymenial layer secreted by paraphyses) but is not an ancestor of the latter. Brief consideration is given to its nutrition and potential position in the phylogeny of the Ascomycota.

Subject areas: palaeontology, mycology

Key words: dispersed cuticle, epihymenial layer, hymenium, septate paraphyses, polyspory, phylogeny

Introduction

The genus *Prototaxites*, whose fossils extend from Silurian (Ludlow) to Upper Devonian strata (Famennian) [1, 2], remains one of the most debated conundra in the Palaeozoic record. Some *Prototaxites* "logs" reached more than one metre in diameter and several metres in height, the maximum being 8.8 m; they were the largest terrestrial organisms of their time [1]. Some, but not all *Prototaxites* axes reveal irregular growth rings. Its habitat has been much debated [3, 4], but based on sedimentological studies *Prototaxites* was found to be truly terrestrial [5]. Being part of terrestrial food chains, its axes have been invaded by boring terrestrial arthropods [6].

Approximately 14 species have so far been described. These have been collected in N-America [1, 7, 8, 9, 10], N-Europe (UK [3, 11, 12], Germany [1, 13, 14, 15, 16, 17]), N-Africa (Libya [18, 19]), Western Asia (Saudi Arabia [20, 21]), and Australia [1]. *Prototaxites* spp. were locally abundant, as concluded from allochthonous charcoalified fragments in Lower Devonian fluvial rocks in the Welsh Borderland, where *Prototaxites* often makes up a high percentage of fossil remains. Eroded, fragmented silicified samples have been found as pebbles in fluvial deposits, e.g. in gravel quarries (Middle Eocene [22] from the Eifel area to coastal Belgium and Netherlands [23]. However, only very rarely were *Prototaxites* samples found with an intact surface layer, exceptions being a few permineralized fossils in the Rhynie chert palaeoecosystem, and fertile parts have been largely missing [10].

As the etymology of the name suggests, its originator Dawson [7] initially identified the large 'trunks' of *Prototaxites* as a conifer with anatomy superficially similar to that in the wood of the extant genus *Taxus* (yew) with its tertiary spirals. Such assignation was subsequently challenged by the phycologist Carruthers [24] who, illegitimately based on precedence, renamed the Lower Devonian Gaspé fossils as *Nematophycus*, here with connotations to

brown algal affinity and resemblance to the laminarialean genus *Lessonia*. Subsequent authors (e.g. Penhallow) [25] supported an algal affinity, while Dawson [26] changed the name to *Nematophyton,* a name, although again illegitimate, was used by a number of authors, including Kidston & Lang [3] (*N. taiti*). Later a red algal affinity was postulated [27], and recently *Prototaxites* axes have been interpreted as rolled mats of liverworts (m~~M~~archantiophytes), which had rolled down a slope and stabilized themselves with their own rhizinae [28, 29]; this interpretation has been rejected [30, 31].

An early dissenting voice had been that of Church [32] who saw resemblances with fungi, a relationship reinforced by Hueber [1]. In a very detailed reinvestigation of Dawson's type, *P. logani*, and more recently acquired material, Hueber described the organism as possessing three forms of interactive hyphae (plectenchyma as opposed to embryophyte parenchyma) with similarities to other *Prototaxites* species.

Definitive evidence for the fungal status of *Prototaxites* and then its position in the fungal clades requires information on its reproduction. Hueber [1] described structures on the margin of a growth increment in *P. logani,* which he interpreted as remnants of a hymenium on a previous outer surface in which dendritically branching hyphae (dendrophyses) were associated with "apparent remnants of primitive basidia in the form of a closely linear or clustered individually inflated sterigmata, each with a prominent speculum" (p. 145; Plate VII). Neither spores nor basal metabasidia were preserved. On such somewhat equivocal evidence, Hueber concluded that a hymenium originally present on the outermost surface of the presumed sporophore (trunk) supported basidiomycete affinity.

Here we describe a different type of hymenium associated with *Prototaxites taiti* in the Rhynie chert [3], which offers an alternative solution to relationships within the fungi. Although the hymenial layer of the type specimen is not in organic continuity with the axis, we are convinced, as were Kidston and Lang [3], that it was neither a parasite nor a

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saprotorophic degrader, but part of *P. taiti*. The new material studied here shows a similar juxtapositioning with *P. taiti* and contains medullary spots. Peels of *P. taiti* comprise detached fragments of the hymenial layer and a larger fragment whose entire surface is covered by a hymenium. Similar organisation detected in charcoalified fragments from the Welsh Borderland leads to the inference that some members of the *Nematothallus* complex, which were common elements of terrestrial ground covers in the Late Silurian-Early Devonian [33], also belong to *Prototaxites.*

Material and Methods

Anatomy was studied using petrographic thin sections and cellulose acetate peels of the silicified matrix:

1) Figured specimen, i.e. thin section N° 2525 from the Hunterian Museum, is part of the original Kidston's collection (Plate X, figures 113,115, 116, 118 in [3]); this section was made by W. Hemingway. Courtesy Dr. Neil Clark.

2) One section and numerous peels from Block 149 in the Lyon Collection at Aberdeen University; thin section 149/CT/B had been prepared by A.G. Lyon in Cardiff and is now in the Aberdeen collection. Courtesy Prof. Nigel Trewin.

Sections had been prepared by standard petrographic techniques. Peels had been prepared by A. G. Lyon by etching smooth surfaces of chert with HF before washing and applying a superficial sheet of cellulose acetate moistened with acetone.

Peels and thin sections were examined in transmitted light using a LEICA DMR microscope fitted with LEICA LAS software, and a Nikon Eclipse LV100ND compound microscope. Confocal images from the thin sections were acquired with a Nikon A1-Si laser-scanning confocal microscope. Autofluorescence of the sample was excited with four laser lines.

Autofluorescence signal was collected with four photomultiplier detectors with the following wavelength emission windows: 425–475 nm for the 405 nm laser, 500– 550 nm for the 488 nm laser, 570–620nm for the 561-nm laser and 675–725 nm for the 640 nm laser. Samples were visualized using a 29.9 µm (1.2 airy units) confocal pinhole and a number of z-stacks (typically between 100 and 400) with optical thickness between 200 and 300 nm each were acquired. The fluorescence signal from each z-stack was then projected onto a maximum projection image.

For SEM investigations coalified material collected at Ludford Corner (Přídolí) and North Brown Clee Hill (Lochkovian) in the Welsh borderland was isolated from the matrix using HCl and HF (protocol in [343, 34]). Selected specimens were mounted on stubs and examined using an environmental scanning electron microscope (ESEM-FEG, Philips XL 30 with field emission gun; FEI).

Results

Light microscopy preparations from Rhynie chert fossils

Figured specimens in same block as the type specimens of *Prototaxites taiti*

Slide Nr. 2525 of the Kidston collection, the petrographic thin section with the type specimen of *Prototaxites taiti* Kidston & Lang 1921 [3], contains an isolated fragment interpreted by Kidston and Lang as its peripheral layer (Fig. 1a). This was based on the presence of medullary spots (figure 1b-d) noted in *P. taiti* and common in other *Prototaxites* species, and the presence of ‘tubes’ within this region thought equivalent to those in the tissues of *Prototaxites*, although with smaller diameter in the peripheral tissue (figures 1a, 2e), and the close proximity to *P. taiti* in the same block. According to Kidston & Lang [3] the peripheral layer, which “has a thickness of about 1 mm” (p. 888), is built up by ‘tubes’ arranged in parallel at right angles to the surface with homogeneous brown contents and fine hyphae in

between these (p. 886). They describe a narrow outer zone above the summits of the vertical tubes as a “structureless, and perhaps mucilaginous, layer during life. It has no sharply defined outer limit, though it is marked as a dark line owing to a granular deposit”.

We interpret this peripheral zone of *P. taiti* as the hymenial layer of an apothecium-bearing ascomycete, the section plane being slightly tangential, the tubes with granular contents being asci with large numbers of small ascospores and the fine hyphae between them as paraphyses. The thin, amorphous and pigmented outermost layer is presumably an amorphous epihymenial layer, the granular deposits therein might have been ~~crystalline~~ secondary metabolites, as secreted by the tip cells of the paraphyses prior to cristallization. Such gelatinous, often brightly pigmented epihymenial layers are common and widespread in the apothecia of extant non-lichenized and lichenized *Pezizomycotina*.

Slide Nr. 149/CT/B, petrographic thin section

In this section (figures 2a, 3-5) several detached fragments of the presumed hymenial layer of *Prototaxites taiti* are preserved. Most of them are tangentially sectioned, but a comparatively large fragment of a median section can be explored (marked in figure 3, white arrow). The hymenial layer is approximately 1100 µm thick. Moreover there are two dark areas contained in this slide with hundreds of sporangia and resting sporangia of a ~~chytrid~~ zoosporic fungus (3~~4~~5). This large assembly probably developed on detritus, as accumulated in the chert palaeoecosystem.

In the thinner part of the median section of the hymenium of *P. taiti*, the asci, paraphyses and epihymenial layers were resolved (figures 4, 5a, b). At maturity the ascus tip reached the surface of the hymenium. The delicate paraphyses (approx. 5-6 µm thick) are septate (figure 4a-e) and end with a globose to pyriform tip cell (figure 4a, d); anastomoses occur (figure 4e). The asci contained more than 100 minute, at presumed maturity well-defined spores with thin,

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translucent wall measuring approx. 6 µm in diameter (figures 4a-c); these are best visible in confocal micrographs (figure 5a-b), the chemical composition of the autofluorescent material being unknown, fluorescence resulting either from mineral or organic preservation. Some of the asci had already released their spores.

The amorphous epihymenial layer of *P. taiti*, with wall fragments of tip cells of the paraphyses and granular deposits embedded therein, was locally peeling off the hymenial surface and detached fragments were found in the surroundings of the hymenia (figure 5d-e). The apical region of asci after spore release appears as holes in these detached epihymenial layers.

Various hyphae of fungal and bacterial invaders are seen below and partly within the hymenial layer of *P. taiti* (figure 5c). They might have grown into the detached hymenial fragments post mortem. Moreover there are many silicate crystals, often rosette-like, around and within the fossils (figure 1a).

Slides 149/B 2, 3, 5, 8 & 9 with peels of *P. taiti*

Serial peels of a block containing *P. taiti* (figure 2a-d) contained several detached fragments of hymenial layers in various section planes (marked with pen in figure 2a), and a near-median section of the hymenial layer covering the surface of an axis (figure 2b, d). A Cross sections of presumed cords are seen (figure 2b, c), but not in conjunction with the axes.

In these serial peels of *P. taiti* two large, dark fragments and a distinctly brighter one are seen (figures 2a, b). The dark fragments lack a peripheral layer, whereas the surface of the bright fragment is covered by an extensive hymenium, the latter being tangentially sectioned. Although this fertile fragment reveals the anatomy characteristic of *Prototaxites* it seems to be more “fleshy”. With high probability the detached hymenial fragments had peeled off such fertile parts when these were either senescent or otherwise damaged. The large number of

detached hymenial fragments, as found in these Rhynie chert specimens, infers an abundance of fertile parts.

Scanning electron micrographs of charcoalified fragments from the Lochkovian Welsh Borderland

Prototaxites sp. (HD(L)171/03)

This small Lochkovian fragment reveals an intact, hummocky surface of the hymenial layer (figure 6a, b) comprising well-spaced structures interpreted as asci. Various developmental stages of asci embedded in the amorphous epihymenial layer indicate that they were ripening at different times (figure 6b). Still closed ascus apices appear near others, which slightly bulge above the surface prior to opening with a slit. After ascospore release the wall of the empty ascus sunk back into the hymenial layer (figure 6b), leaving a roundish slit before getting covered by mucilage. Groups of ovoid, somewhat shrivelled presumed ascospores were repeatedly seen lying on the hymenial surface (figure 6d); their size (approx. 5.5 µm long) corresponds to those seen in the silicified specimens (figure 4). Some of these presumed ascospores seemed to germinate (figure 6e), but cannot be distinguished from contaminant fungal spores. Both landing of ascospores and their germination on the hymenial surface occur frequently in extant ascomycetes. However, crystals of the same size and shape as the presumed ascospores are frequently found on this type of material.

On the lower surface of this fragment are medullary spots embedded within hyphae (figure 6c). The epihymenial layer has locally peeled off (figure 7a), revealing a reticulum characteristic of cuticles of the *Nematothallus* complex. The probably largely immature asci are well preserved (figure 7a-c), but the most of the delicate paraphyses were lost, probably during charcoalification, leaving some impressions on the surface of the asci (figure 7c). Presumed paraphyses are seen in figure 7c. The amorphous epihymenial layer is well

preserved, revealing impressions of tip cells of paraphyses on its inner surface; these are visible at sites where the epihymenial layer detaches (figure 7a). A subhymenial layer with crozier formation was not seen here nor in the silicified material examined by light microscopy. As described by Kidston and Lang [3] for the latter "the tubes bend outwards and branch, and in the superficial layer … stand parallel at right angles to the surface" (p.888). This specimen was invaded by either fungal or bacterial contaminants (figure 7b).

A charcoalified apothecial fragment of a presumed pPezizomycete from the Late Silurian Shropshire

The unnamed specimen LL03/02 from Ludford Lane, Shropshire, is interpreted as a fragment of at least two apothecia, which had been growing side by side (figure 8a, b, e). In contrast to the hymenium of *Prototaxites taiti,* a dense subhymenial layer is seen with presumed crozier formation (figure 8c). The apothecia have a thin margin built up by only one cell layer (figure 8b, e). The apex of the polysporous ascus reached the hymenial surface at maturity (figure 8e, f). Some of the tip cells of the septate paraphyses bend and branch (figure 8f, g), a feature which is seen also among some extant ascomycetes. This specimen, which was unfortunately lost while being turned over for additional fracturing and investigations, represents the first apothecium-bearing member of the *Pezizomycotina* so far found.

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Discussion

***Prototaxites taiti*: an ascomycete with extensive hymenial layer**

A striking structural similarity of the ascomata of *Prototaxites taiti* with the sori (meiosporangia-bearing zones) of the large sporophytes of marine brown algae (kelps) of the order *Laminariales (Phaeophyceae, Heterokonta)* is evident. Sori of *Laminariales* are formed as patches on the surface of the phylloids ("blades"); they are built up by sterile filaments (paraphyses) interspersed with elongate,

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ascus-shaped meiosporangia comprising large numbers of biflagellate zoospores. The morphological similarity of *Prototaxites* stems with cauloids and of their basal part with holdfasts of *Laminariales* was reported by earlier investigators [13, 17, 18, 24, 25]. However, the meristem-derived tissues of laminarialean sporophytes differ anatomically from the plectenchyma of *Prototoaxites* spp. Fossil records are largely missing. Based on multigene phylogenetic analyses the branching time of *Laminariales* from *Ectocarpales* (filamentous brown algae) is estimated around 100-90 Ma ago [36]. Thus a laminarialean affiliation of the genus *Prototaxites* can be excluded.

Based on the present findings *Prototaxites taiti* is interpreted as a basal ascomycete; in the fertile state a hymenial layer comprising polysporous asci and delicate, septate paraphyses covered the surface of either part of its axis or of fertile, presumably lateral and/or terminal outgrowths. It is not clear how the hymenial fragment of the type specimen of *P. taiti* (figure 1a) was connected to the axis; the "fleshy" fertile fragment in the peels (figures 2a, b) might represent a fruit body as a lateral outgrowth of the axis. Further material is needed to answer these questions.

The oldest ~~fruit bodies~~ ascoma of an ascomycete so far described are the beautifully preserved perithecia of *Palaeopyrenomycites devonicus* ~~Taylor, Hass, Kerp, Krings et Hanlin 2005~~ [3~~6~~7, 3~~7~~8], a presumed ~~S~~sordariomycete, parasite or epibiont of *Asteroxylon mackiei*. However, the most basal taxa among the extant *Pezizomycotina* (i.e. the *Peziomycetes* and *Orbiliomycetes*; [3~~8~~9, ~~39~~40]), produce neither ostiolate peri- or pseudothecia, nor closed cleisto- or gymnothecia, but apothecia, the former ascomal types having evolved independently in diverse taxa [~~4~~01-4~~3~~4].

The dimensions of the hymenial layer of *Prototaxites taiti* differ considerably from extant ascomycetes, both in circumference and height. Very large apothecial surfaces are found, e.g., among the earth tongues (*Geoglossaceae*, *Geoglossomycetes*; figure 9b), the elfin saddles (*Helvellaceae*, *Pezizomycetes*) and the morels (*Morchellaceae*, *Pezizomycetes*). Their comparatively soft, large ~~fruit bodies~~ ascomata are relatively short-lived (few weeks) and die

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off after ascospore release, all asci reaching maturity at almost the same time. In contrast, the tough, club-shaped Dead Moll's Finger (*Xylaria longipes*) and related taxa among the *Xylariaceae* as well as Caterpillar Fungi (*Cordiceps* spp., *Sordariomycetes*) have no superficial hymenial layer, but large numbers of minute perithecia peripherally inserted in their robust sclerotia. Long-living hymenial layers are found in the apothecia of the majority of lichen-forming ascomycetes, especially in the *Lecanoromycetes* (e.g. *Cladonia* spp., *Cladoniaceae*, or *Baeomyces* spp., *Baeomycetaceae*; figure 9c, d), their fruit bodies producing asci over many months to few years. However, their apothecia are minute compared with the aformentioned taxa.

The hymenial layer of *Prototaxites taiti* is approx. 1100µm high and thus more than twice the size as reached by extant apothecia-bearing ascomycetes. The length of the mature ascus, as recorded in the taxonomic literature, does not necessarily reflect the full height of the hymenium; the apex of the mature ascus may be below the hymenial surface and the epihymenial layer often accounts for additional micrometres. In extant ~~Pe~~pezizomycetes, ascus lengths around 300-380 µm are found in morels (*Morchella* spp.), up to 390µm in Cedar Cup (*Sepultaria sumneriana* ~~(Cke) Mass.~~), and up to 450µm in Midnight Disco (*Pachyella violaceonigra* ~~(Rehm) Pfister~~), all representatives of the *Pezizales*/*Pezizomycetes* (data from [4~~4~~5]).

The oldest fossil apothecia

This is the first report on fossil apothecia, the characteristic ~~fruit bodies (~~ascomata~~)~~ as formed by numerous taxa within the subphylum *Pezizomycotina*, which are considerably older than those found in Cenozoic amber. So far a striking discrepancy between the presumed age of the earliest apothecia-bearing ascomycetes (Ordovician) as concluded from molecular data sets [3~~7~~8, 3~~8~~9], and the fossil record was evident. So-called calicioid ascomata (a paraphyletic

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assemblage of taxa with stalked apothecia and superficial mazaedium, i.e. a powdery spore mass, as released by deliquescent asci [4~~5~~6, 4~~6~~7]), were found in Cenozoic amber [4~~7~~8-5~~0~~1].

Extant representatives of the Pezizomycotina have a haploid vegetative mycelium. ~~Fruit body~~ Ascoma formation is initiated by dikaryon formation, either via spermatisation of ascogonia (the contact sites being thin-walled trichogynes, which grow out of the ascogonia) or via fusion of non-differentiated vegetative hyphae (somatogamy). Upon successful dikaryotisation a system of dikaryotic hyphae grows towards the future hymenial layer of the ~~fruiting body~~ascoma. The haploid hyphal system in close contact with the ascomal primordium builds up the ~~fruiting body~~ ascoma with characteristic margin and the hymenial layer with paraphyses, which, in many groups, secrete the hydrophilic hymenial gelatine and the epihymenial layer plus the pigments located therein. In the majority of *Pezizomyc~~e~~otina*, crozier formation with nuclear migration into the penultimate cell along the dikaryotic ascogenous hyphae, precedes karyogamy; this allows the maintenance of the dikaryotic state with nuclei of opposite mating types.

***Neolectomycetes* (*Taphrinomycotina*): basal *Ascomycota* with club-shaped ~~fruit bodies~~ascomata**

The *Taphrinomycotina*, formerly termed *Archaeascomycetes*, is the most basal out of three subphyla of the *Ascomycota*; it comprises the classes *Taphrinomycetes* (plant pathogens), *Schizosaccharomycetes* (fission yeasts), and the monotypic class *Neolectomycetes* ~~O.E. Eriks. & Winka 1997~~ (with one order, one family and one genus, *Neolecta* Speg., comprising four presumably saprotrophic species) [5~~1~~2]; these are the only extant representatives of the *Taphrinomycotina* which form ~~fruit bodies~~ascomata.

The stipitate, clavate ascomata of *Neolecta* spp. are covered by a coloured hymenial layer (figure 9a), no ascomal margin being differentiated. The vegetative hyphae are binucleate, the

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ascogenous hyphae multinucleate, the nuclei being haploid [5~~2~~3]. Only when asci are to be formed in *Neolecta* spp. pairs of nuclei, presumably of opposite mating types, are formed and segregated via septum formation from the rest of the hypha [5~~2~~3]; a dense subhymenial layer is missing and no croziers are formed. The mating system of *Neolecta* spp. has not yet been genetically analysed, and it is not known how the pairing of nuclei is achieved prior to karyogamy and meiosis. In the more advanced, plant pathogenic *Taphrinomycetes* (*Taphrina* spp.) the dikaryon is formed soon after ascospore germination via fusion of budding cells, only the dikaryotic (bi-nucleate) mycelium being infective. Based on molecular data sets [5~~3~~4, 5~~4~~5] *Neolecta* (i.e. the *Neolectomycetes*) was referred to as «a fungal dinosaur» [5~~5~~6].

***Prototaxites taiti*: a basal ascomycete**

According to the present findings *Prototaxites taiti* represents a basal ascomycete which combines characteristic features of *Neolectomycetes*/*Taphrinomycotina*, such as asci without crozier formation (in addition to the SEM specimen several millimetres of hymenial length were studied in petrographic thin sections), their inoperculate apex opening with a slit, and of *Pezizomycotina*, i.e. a system of paraphyses which secrete mucilage into the hymenial and epihymenial layer. However, Upper Silurian and Lower Devonian *Prototaxites* spp. -were unlikely ancestors of the *Pezizomycotina* since they were contemporaries of apothecia-bearing ascomycetes with more advanced character traits. The unnamed Upper Silurian apothecial fragment (LL03/02) with a thin apothecial margin (figure 8b, e), distinct paraphyses and polysporous asci, as shown in figure 8, reveals a dense subhymenial layer (figure 8d) and presumed croziers (figure 8c), both being characteristic features of *Pezizomycotina*. No croziers could be resolved in *Palaeopyrenomycites devonicus* [3~~6~~7, 3~~7~~8].

How does our interpretation of *Prototaxites taiti* as a basal ascomycete correlate with Francis Hueber's [1] emendation of the genus *Prototaxites* as a representative of the class *Agaricomycetes* (=*Holobasidiomycetes*)? Based on detailed investigations of *P. loganii*

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samples, Hueber interpreted the tangentially sectioned peripheral layer as a hymenial layer and some of the structures therein as sterigmata of presumed basidia. Neither basidia proper, nor basidiospores were seen in this sample. The presumed sterigmata could be remains of ascus walls in the tip region after spore discharge. Median sections are required to solve this problem.

In the neotype of *P. loganii* Hueber [1] described a cluster of very thin, finely branched presumed dendrophyses (= dendrohyphidia), a type of sterile hyphae as found in the hymenia of some basidiomycetes. No comparable structures were found in *P. taiti*. Since the hymenium, as the peripheral layer, is also easily accessible to fungal and bacterial colonizers during life time and post mortem (figure 5c) and crystallization products of minerals may have developed during the fossilisation process on or near the hymenial surface, a more detailed analysis of the cellular structure of the dendrophyses would be of interest.

The septal pore apparatus, a phylogenetically informative character trait, has been investigated in *P. southworthi* with TEM techniques [5~~6~~7]. It shows a quite complex central pore with surrounding material, but not a dolipore as typically found in extant *Agaricomycetes*. A surprisingly similar situation was observed in *Neolecta vitellina* [5~~7~~8], whose septal pore apparatus differs structurally and biochemically from the one as typically found in *Pezizomycotina*. The HEX-1 protein, as contained in the membrane-bound Woronin bodies which are plugging the septal pore of *Pezizomycotina* [5~~8~~9], is missing in *Neolecta* [~~59~~60], but a unique type of material is present. Healy et al. [5~~7~~8] conclude that the septal pore-occluding structure in *Neolecta* might have evolved independently.

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Polyspory

With regard to the current discussion about the evolution of polyspory among the *Pezizomycotina* [6~~0~~1] it is particularly interesting to see polysporous asci in the perithecia of

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Palaeopyrenomycites devonicus (up to approx. 16 ascospores per ascus; [3~~6~~7, 3~~7~~8]), in the earliest yet found apothecium from the Upper Silurian (present study) and in *Prototaxites taiti* (present study).

The majority of extant representatives of the *Pezizomycotina* contain eight ascospores per ascus (octospory), these being the result of one mitosis following meiosis. However, polyspory is widespread, some taxa containing more than 100 spores per ascus. Examples are > 500, as in *Melanophloea* (lichenized ascomycete *incertae sedis*; [6~~1~~2]), or > 1000 as in *Brigantiaea* (*Lecanorales*) and *Gyalidea* (*Ostropales*; both *Lecanoromycetes*). Among extant *Pezizomycotina* polyspory occurs in *Eurotiomycetes*, *Dothideomycetes*, *Lecanoromycetes*, *Leotiomycetes*, *Lichinomycetes*, *Orbiliomycetes*, *Sordariomycetes*, often punctually (one or few polysporous among many octosporous species per genus), but sometimes prominent, with the majority of species per genus or even family being polysporous. Polyspory is also widespread within the *Taphrinomycotina*, the most basal of all subphyla of *Ascomycota*.

Some authors distinguish between true polyspory when asci contain >100 spores, and non-true polyspory for taxa with more than eight and less than hundred spores per ascus [6~~0~~1]. Polyspory results from either several mitoses following meiosis (probably the most common case), from multiple budding (e.g. in various *Taphrina* sp., sometimes also in *Neolecta* spp; [5~~2~~3, 6~~4~~5]), from conidium formation by ascospores within the ascus (e.g. in *Rhamphoria* [*Sordariomycetes*], *Brigantiaea* and *Gyalidea* [*Lecanoromycetes*]), or from breaking apart of multicellular ascospores within the ascus (e.g. various species in the genera *Claussenomyces*, *Tympanis* [*Leotiomycetes*] or *Cordyceps* [*Sordariomycetes*]).

Among the lichen-forming *Pezizomycotina* polyspory is widespread [6~~0~~1, 6~~1~~2]. Few families such as the *Ascosporaceae*, *Biatorellaceae* (*Lecanoromycetes*) and *Thelocarpaceae* (incertae sedis) and at least 16 genera therein are largely polysporous, while 38 (unrelated) genera comprise only few polysporous species (representatives of *Lecanoromycetes*, *Lichinomycetes*,

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Eurotiomycetes). Polyspory was assumed to have evolved independently at least 57 times among extant lichen-forming ascomycetes [6~~1~~2]. However, the possibility of polyspory being an ancestral trait and repeatedly lost during evolution cannot be excluded. Larger sets of molecular data and distinctly more fossil records are required.

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The detached epihymenial layer of apothecia: a type of "dispersed cuticle"

In the palaobotanical literature the term cuticle is used for (usually fragmented) surface layers of various origins: arthropods, plants, or thalli of presumably lichenized fungi. Dispersed cuticles of nematophytes had so far been interpreted as top layer of vegetative thalli [6~~2~~3, 6~~3~~4]. Here we present the first examples of dispersed cuticles as a detached epihymenial layer of apothecia in permineralized *Prototaxites* aff. *taiti* from Rhynie chert material and from charcoalified *Prototaxites* sp. from the Lochkovian of the Welsh Borderland. Apothecia might be more common in the fossil record than previously assumed, e.g. among nematophytes with distinct palisade zone below the peripheral layer. Asci differ in their cell wall structure, composition and function from paraphyses and vegetative hyphae (figure 10a-c) and thus might often have been lost during fossilisation.

Looking at extant apothecia-bearing ascomycetes one should keep in mind that their vegetative mycelium is either hidden in the substrate (saprotrophs) or in a host tissue (parasites) or ensheathing plant root systems (mycorrhizae), only their ascomata being visible above ground. On suitable substrates, apothecia are locally abundant and appear in large and often colourful clusters (e.g. in the genera *Sepultaria*, *Peziza*, *Helotium*, *Hymenoscyphus* etc., examples in [4~~4~~5]). Upon suitable fossilisation fragments of apothecial discs should be detectable in the fossil record. Exceptions are the lichenized taxa; these have to expose their photobiont cell population to adequate illumination and therefore differentiate their vegetative thallus at or above ground (rock, soil, bark etc.), fossilised remains of their vegetative thallus

having been found from the mid-Palaeozoic onwards [33, 3~~5~~4], but fertile fragments remain to be found.

Nutritional aspects

Assignation to the heterotrophic fungi of organisms with such enormous body mass ('trunks' of Saudi Arabian *Prototaxites* attained a length of 8.8m and a diameter of 1.2m, figure 14 in [1]) raises questions on sources of nutrients which Hueber suggested were acquired via a hypothetical extensive soil mycelium exploiting decaying organic material. Our present data do not contribute substantially to our understanding of its nutritional strategies. There is no evidence for underground structures in *Prototaxites,* although the basal part of *P. hefteri* resembles the basal part of *Laminaria* sp. whence rhizoids derive [17]. Edwards and coworkers described putative cords or rhizomorphs of *Nematosketum*, closely allied to *Prototaxites* [6~~5~~6] and presumed rooting structures of *Prototaxites* sp. in rocks of the Early Devonian Anglo-Welsh Basin [6~~6~~7]; similar structures likely occur alongside sections of *P. taiti* (this investigation, figure 2c). The authors speculated that such organs might have translocated soluble carbohydrate produced by leakage from biofilms or their decay. A similar source has been suggested from $\delta^{13}C$ data from *Prototaxites* and coeval vascular plants [6~~7~~8]. The former showed a considerable range in values (-15.6% to -26.6%) allowing the inference of heterotrophic nutrition involving a number of isotopically distinct substrates. The values determined by Graham *et al*. [29] were more consistent with those obtained experimentally from *Marchantia*, but no allowance was made for differences in atmospheric $\delta^{13}C$ in Devonian times. They used such data to support their hypothesis that *Prototaxites* represented accumulations of concentric layers of thalloid hepatic mats, bound together by rhizoids, procuced on rolling down a slope. Both isotopic data and marchantialean affinity have subsequently been questioned and concluded unlikely on sedimentological, anatomical and geochemical grounds (e.g. [30, 31, 6~~7~~8, 6~~8~~9]).

Saprotrophic, lichenised or mixed nutritional lifestyle?

Based solely on energy relationships, Selosse [~~68~~70, 71] had suggested a lichen-type association in *Prototaxites*, an affinity reinforced on very tenuous anatomical grounds by Retallack and Landing [10], but a sufficiently large and productive photobiont cell population has not yet been convincingly demonstrated. However, as in the other genera of nematophytes [33] the photobiont cell population might not have been preserved during fossilization. Almost all green algal and cyanobacterial cells in the Lower Devonian lichens *Chlorolichenomycites salopensis* and *Cyanolichenomycites devonicus* were missing, but mucilaginous cyanobacterial sheaths were well preserved [3~~5~~4].

When part of the *Prototaxites* axes were covered by a hymenial layer, as concluded from the present findings, lichenized zones might have been either interspersed with fertile areas, or restricted to either the surface proper or lateral outgrowths of a non-fertile basal part, as in podetia of cup lichens (*Cladonia* spp., figure 9d). Alternatively the basal cords of *Prototaxites* might have connected the fertile stems with the common and widespread, possibly lichenized nematophytes with dorsiventrally organized thalli, common and widespread organisms in the ground cover of terrestrial ecosystems of the Mid-Palaeozoic [33, 66]. This situation, although in miniature format, is found in extant *Dibaeis* and *Baeomyces* spp. (figure 9c), but also in lichenized basidiomycetes (*Agaricomycotina*) with non-lichenized ~~fruit bodies~~ basidiomata (e.g. *Lichenomphalia* spp.~~:~~ [7~~0~~2]). However, a superficial hymenial layer and an underlying, photosynthetically active photobiont cell population are not mutually exclusive; this situation occurs in innumerable representatives of *Lecanoromycetes* with a lichenized, so-called thallus margin around the apothecial disc (e.g. *Parmeliaceae*, *Xanthoriaceae*), photobiont cells living not only in the lateral margin, but also underneath the hymenium, the latter allowing sufficient light transmission in the hydrated state, especially when ascospores are hyaline. If originally

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present below the hymenial, layer traces of green algae or cyanobacteria should have been fossilised in the Rhynie chert material of *Prototaxites taiti*.

It is also possible that *Prototaxites* had a mixed nutritional life style with simultaneous saprotrophic and symbiotic acquisition of fixed carbon. The majority of extant lichen-forming ascomycetes are physiologically facultatively biotrophic and thus can be axenically cultured in the aposymbiotic state. In nature many lichenized taxa likely derive nutrients not only as soluble, mobile photosynthates from their photoautotrophic partner, but also saprotrophically, e.g. by enzymatic degradation of organic matter such as the cellulosic mother cell walls of green algal photobionts after autospore formation or decaying plant material in the substratum. Hyphae of *Icmadophila ericetorum*, a lichen-forming ~~L~~lecanoromycete growing on rotten wood (lignin having largely been removed by white rot fungi), were shown to grow through the cell walls of woody tissues which had been partially degraded [7~~1~~3]; quantitative data are missing. The large axes of *Prototaxites* spp. certainly carried a diverse and probably at least partly beneficial microbiome which remains to be circumscribed, epi- and endolichenic bacteria and fungi having been found in the Lower Devonian *Chlorolichenomycites salopensis* [7~~2~~4].

Fossil records and the calibration of the molecular clock

Prior to the advent of molecular genetic tools the shape and ontogeny of the fruiting bodies, together with ascus types, were prime characters in ascomycete classification. However, in molecular phylogenies both character sets turned out to be homoplaseous; thus, groupings such as d~~D~~iscomycetes (comprising apothecia-bearing taxa), p~~P~~yrenomycetes (comprising perithecia-bearing taxa) etc., as found in the older literature, became obsolete [4~~0~~1-4~~3~~4]. Phylogenetic data sets indicate that ascomycetes derived from other fungal lineages in the Proterozoic, estimates ranging between 1490 and 600 Myr, depending on calibration [~~39, 45, 73-79~~40, 46, 75-81]. Unfortunately the fruiting bodies of the majority of ascomycetes lack

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robust structures capable of withstanding microbial degradation and thus are very rarely found in fossil records. Well-preserved, fertile fungal fossils are urgently needed for calibrating the molecular clock. To conclude: the beautifully preserved Rhynie chert material and the charcoalified specimen from siltstone of the Welsh Borderland, as investigated in this study, give new insights in the taxonomic affiliation of the genus *Prototaxites*.

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Figure legends

Figure 1. Petrographic thin section of *Prototaxites taiti* in bright field (a) and confocal laser microscopy (b-c), and peel in bright field microscopy (d). (a-c) the type specimen of *P. taiti* from the Rhynie Cherts, new photograph of slide Nr. 2525 of the Kidston Collection. (a) this fragment is depicted in black and white in Kidston & Lang (1921) [3] in figure 113, with the following legend: „View of one of the sections of the second fragment which shows the peripheral tissues“. Here these peripheral tissues are interpreted as the hymenial layer of the ascoma of an ascomycete, the section plane being slightly tangential. Note the inumerable

silicate crystals, best visible outside the tissues. (b-d) Medullary spots : in confocal laser microscopy (b,c), (slide Nr. 2525), intense wefts of hyphae in close contact with a population of minute cells visible in bright field microscopy (d), (slide 149 B 3).

Figure 2. Slides of a petrographic thin section and a series of peels of *Prototaxites taiti,* as prepared by Prof. A.G. Lyon. (a) Overview of petrographic thin section 149 CT B (see details in figures 3-5) and serial peels in slides 149/B 2, 3, 8 & 9. (b) detail of slide 149/B 8, the position of figures (c) and (d) being marked. (c) cross-section of a cord (or a very young axis?) with a somewhat denser peripheral zone. (d) slightly tangential section of the peripheral layer of a »fleshy» axis which, in the present study, is interpreted as the hymenial layer of an apothecium-bearing ascomycete. (e) confocal laser micrograph of the hymenial layer of the type specimen of *P. taiti* (slide Nr. 2525 of the Kidston and Lang collection, see Fig. 1(a), showing the tubes, i.e. asci with ascospores, in the hymenial layer.

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Figure 3. Petrographic thin section of *Prototaxites taiti* from Rhynie chert specimen Nr. 149 CT B shown in figure 2a. The white arrow points to a median-longitudinally sectioned fragment of the presumed hymenial layer, black arrows to additional hymenial fragments; framed is a thin area of which photographs in figures 4 and 5 had been taken. The asterisk refers to part of a thin, cross-sectioned axis of *Prototaxites* with a simple peripheral zone. Small dots refer to areas with hundreds of sporangia of a ~~chytrid~~ zoosporic fungus (insert: resting sporangium; see [35]). Photo: Thomas Honegger.

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Figure 4. (a-e) *Prototaxites* aff. *taiti*, details from the longitudinal section in slide Nr. 149 CT B (see Fig. 3). Polysporous asci and thin-walled, delicate paraphyses. The darkly pigmented epihymenial layer is best visible in (d). The arrow in (e) points to anastomosing paraphyses.

Same magnification in (a-e).

Figure 5. *Prototaxites* aff. *taiti*, details from slide Nr. 149 CT B; (a-b) confocal laser microscopy, (c-e) bright field light microscopy. (a) Detail of the hymenial layer with asci, paraphyses and invasive, contaminating fungal filaments. (b) Spores inside an ascus. (c) Contaminating fungal and ~~actino~~bacterial filaments growing below the hymenial layer. (d) Laminal view, and (e) tangential (left) and cross section (right) of detached fragments of the epihymenial layer, with tip cells of paraphyses and open ascus tips, the interspace being filled with dark pigments ~~and/or minute pyrite crystals~~.

Figure 6. SEM micrographs of a charcoalified fragment of the hymenial layer of *Prototaxites* sp. (HD(L)171/03). (a-b), (d-e) laminal view of the surface. (a) Overview; (b) Detail of the hymenial surface. The asterisks mark an ascus apex prior to dehiscence. Thick arrows point to asci shortly after ascospore release, the inoperculate ascus tips having slightly expanded and opened with a slit. Thin arrows point to empty asci whose wall sank back into the hymenial layer. (c) Lower surface of the specimen, (d) Arrows point to shrivelled, presumed ascospores after landing on the hymenial surface. (e) Presumed ascospores having germinated next to the apex of the empty ascus.

Figure 7. SEM micrographs of the charcoalified fragment of the hymenial layer of *Prototaxites* sp. (HD(L)171/03), lateral view. (a) The dense epihymenial layer is partly peeling off (see figure 5a-b). (b). Detail of the hymenial layer with immature asci. Arrows point to presumed fungal or ~~actino~~bacterial invaders. (c) Remains of a few thin-walled paraphyses. The asterisk points to an ascus with superficial impressions of lost paraphyses.

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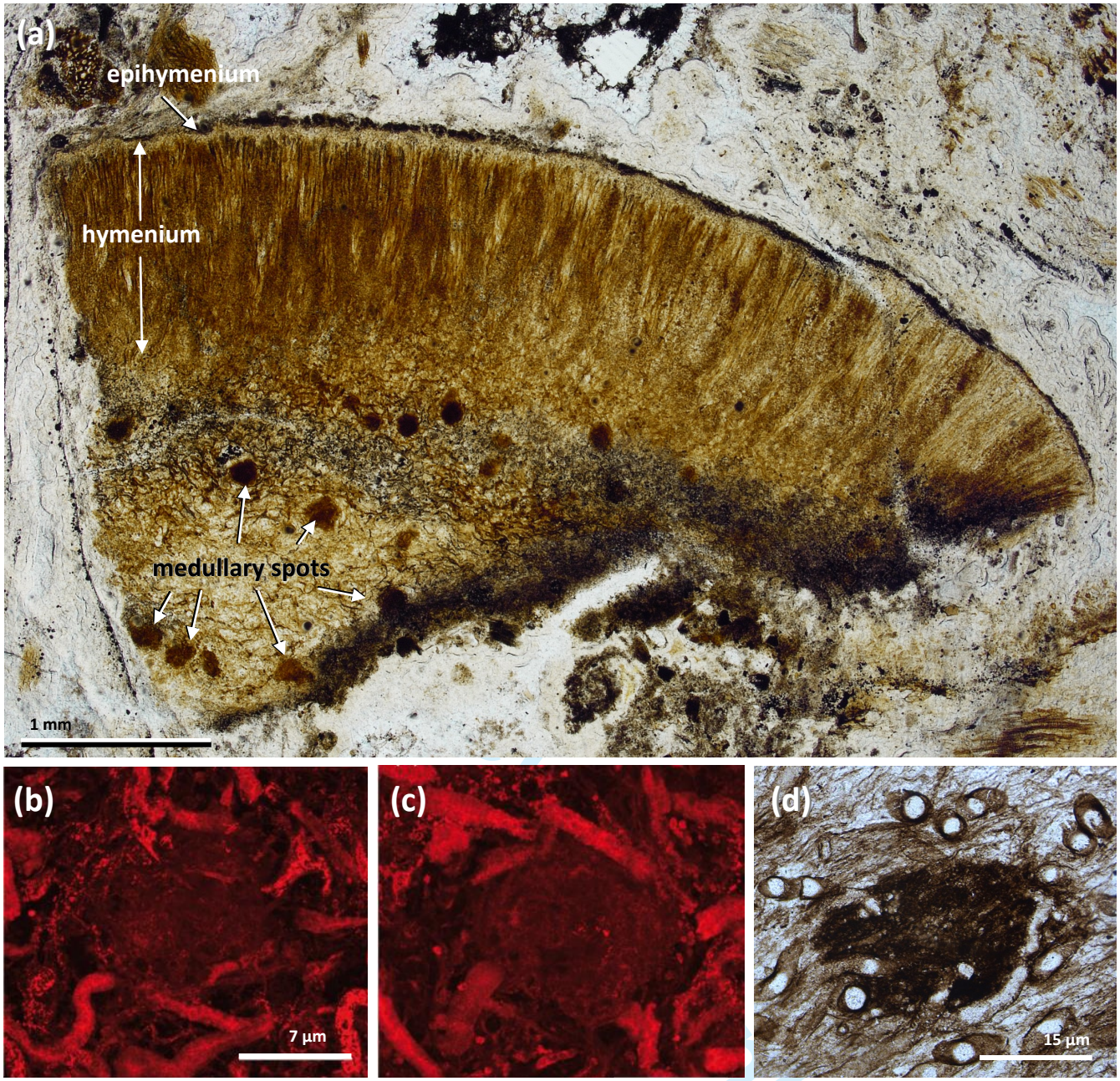
Figure 8. SEM micrographs of a charcoalified apothecial fragment from the Late Silurian

(LL03/02). (a) Upside - up, and (b), (e) upside - down positional views of the specimen. (c) Presumed crozier in the basal part of the hymenial layer. (d) The longitudinally fractured hymenial layer is built up by slim, unbranched paraphyses. The numerous ascospores within the fractured, polysporous ascus reveal irregular outlines, i.e. are deformed. Arrows point to ~~actino~~bacterial contaminants which had grown post mortem over the fragment . (e) Detail of surface in (b): The thin apothecial margin is built up by parallel hyphae. (f) The rounded tip cells of parpaphyses are grouped around presumed ascus apices. (g), Septa are visible in fractured paraphyses.

Figure 9. Extant ascomycetes with clavate to roundish ~~fruit bodies~~ sporophores (ascomata). (a) The presumably saprotrophic *Neolecta vitellina*, one out of 4 species of Neolectomycetes, the only Taphrinomycotina with ascomata. The extensive hymenial layer is bright yellow. Photo courtesy of Raymond McNeil. (b) The saprotrophic Dark-Purple Earth Tongue (*Geoglossum atropurpureum,* syn. *Thuemenidium a.*). Photo courtesy of Knud Knudsen. (c) The stalked, non-lichenized ascomata of *Dibaeis baeomyces* (syn. *Baeomyces roseus*) growing out of the crustose lichen thallus. (d) Convex ascomata of *Cladonia bellidiflora* with red colouration (due to crystals of the anthraquinone bellidiflorin in the epihymenial layer) on the erect podetium whose surface is covered by dorsiventrally organized, lichenized squamules.

Figure 10. Light micrographs of iodine-stained free-hand sections of the ~~fruit body (~~ascoma~~)~~ (apothecium) of extant *Peltigera* spp. (lichenized ascomycetes) whose ascus wall differs structurally and chemically from ascospores, paraphyses and vegetative hyphae. (a) Detail of the hymenial layer of *P. canina* and (b) mature ascus with 8 spindle-shaped, 3-septate ascospores, both stained with Lugol‘s solution (IKI). (c) Detail of the hymenial layer and adjacent margin of *P. venosa* subjected to the van Wisselingh test for chitin (red colouration of chitosan after hydrolytic transformation of chitin). Red colouration, indicative of chitin, is

discernible in the walls of vegetative hyphae of the apothecial margin, of paraphyses and of the ascospores. In both preparations the ascus wall stains blue with iodine due to amyloid compounds (presumably lichenin-like glucans with β-1,3 and β-1,4 linkage bonds).


(a)
epihymenium
hymenium
medullary spots
1 mm
(b)
7 µm
(c)
(d)
15 µm

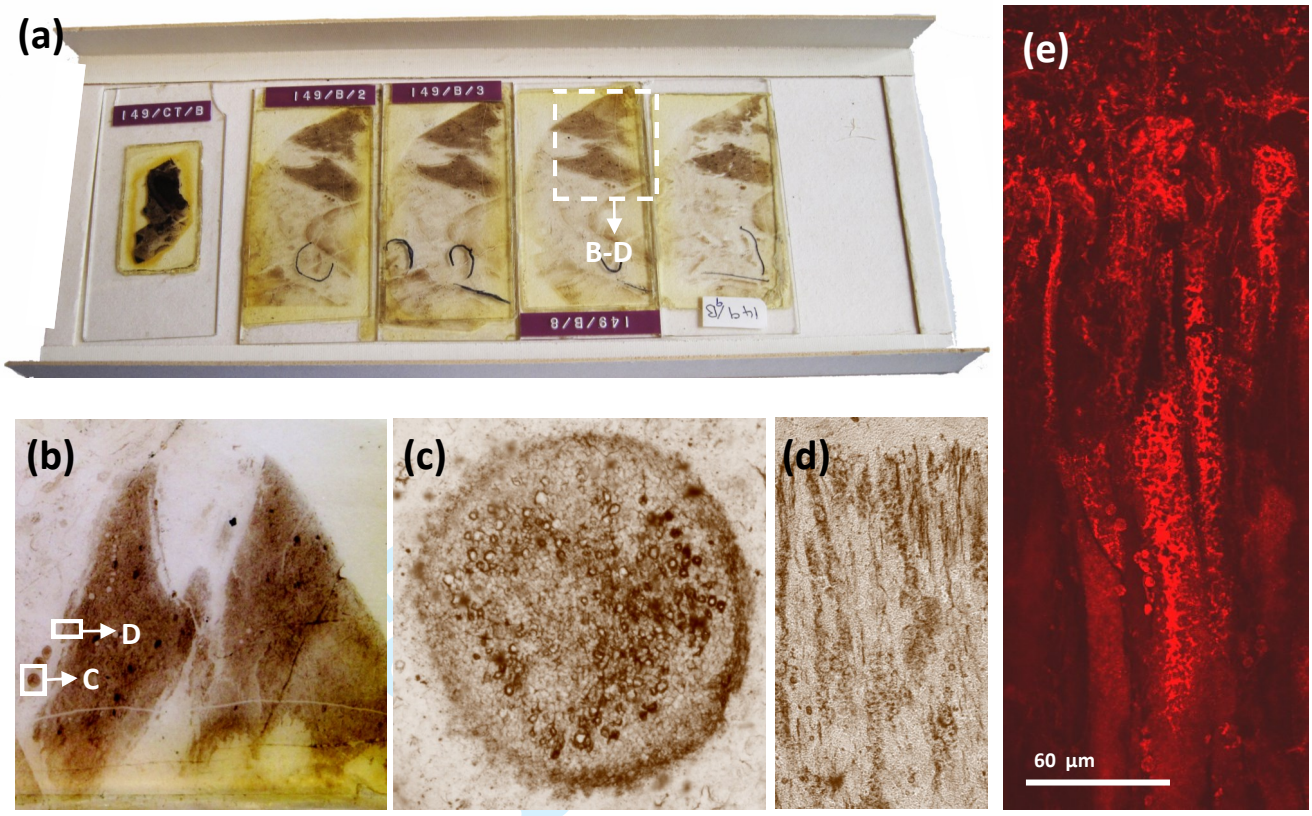
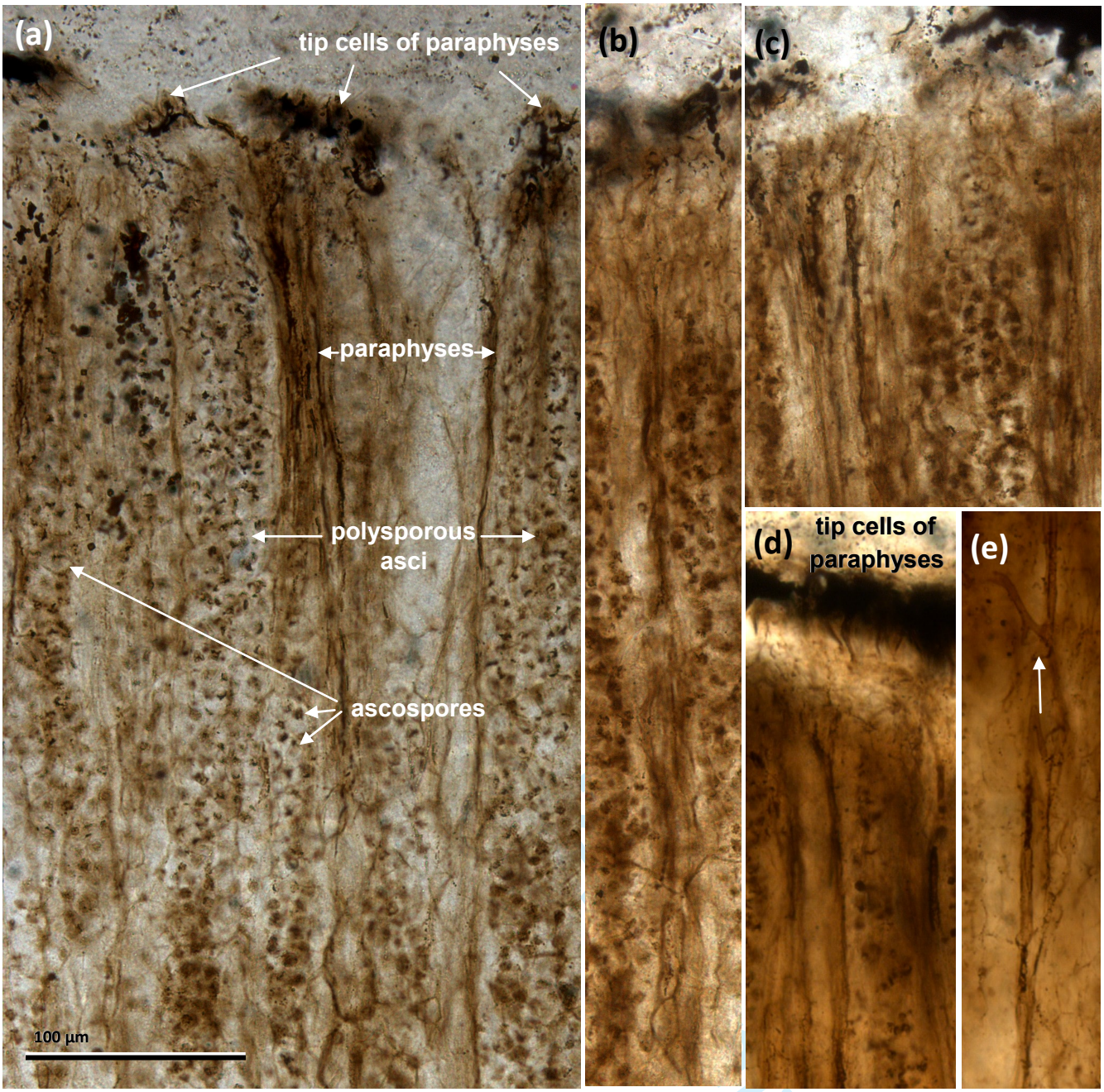
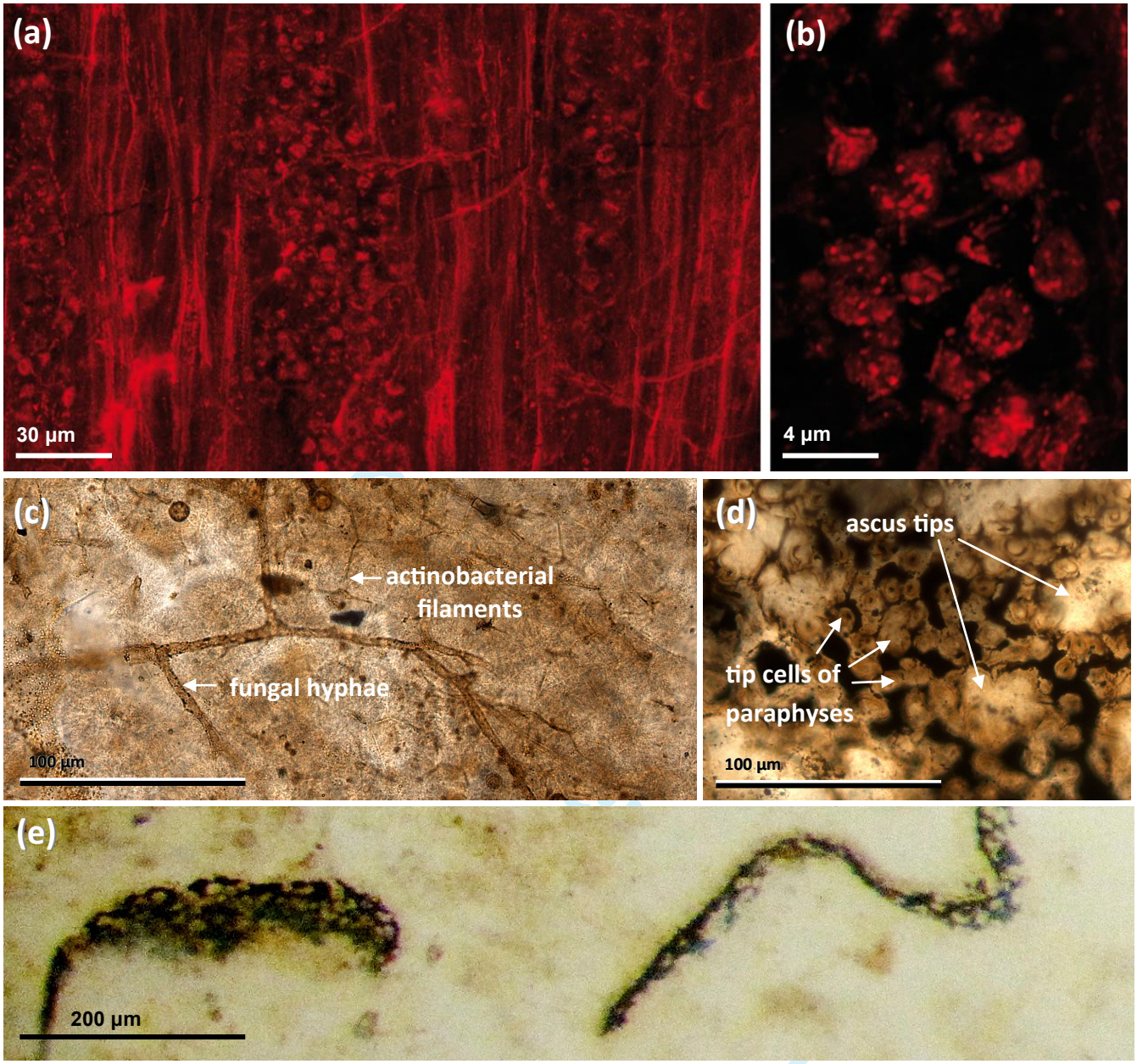
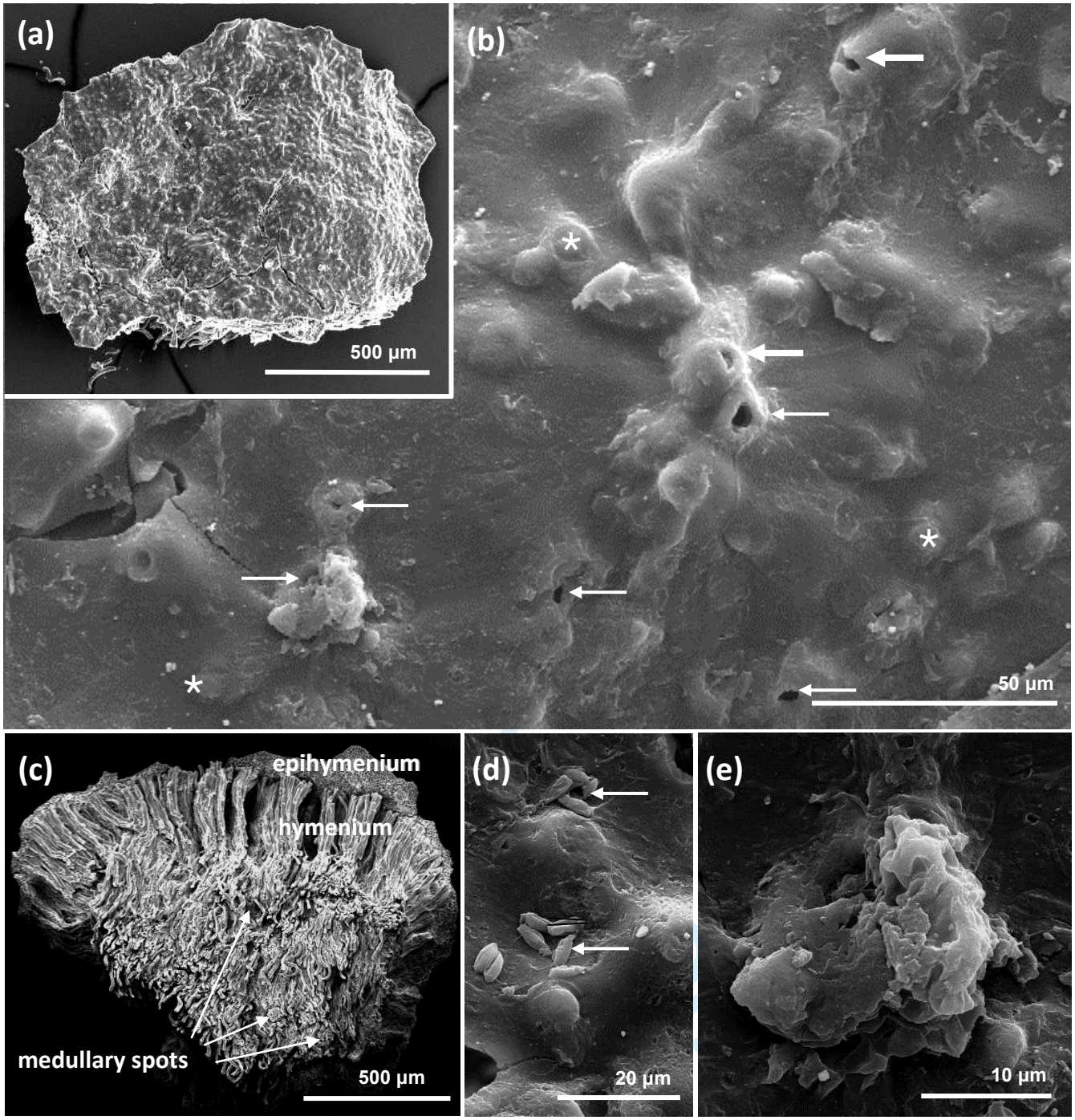
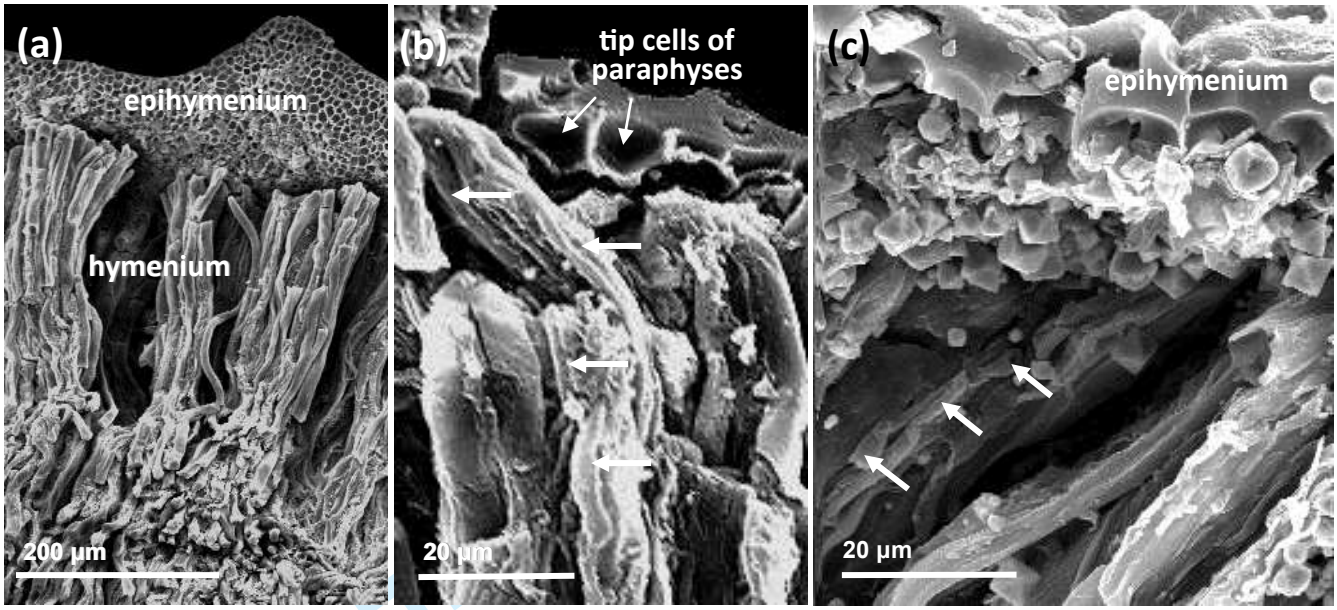


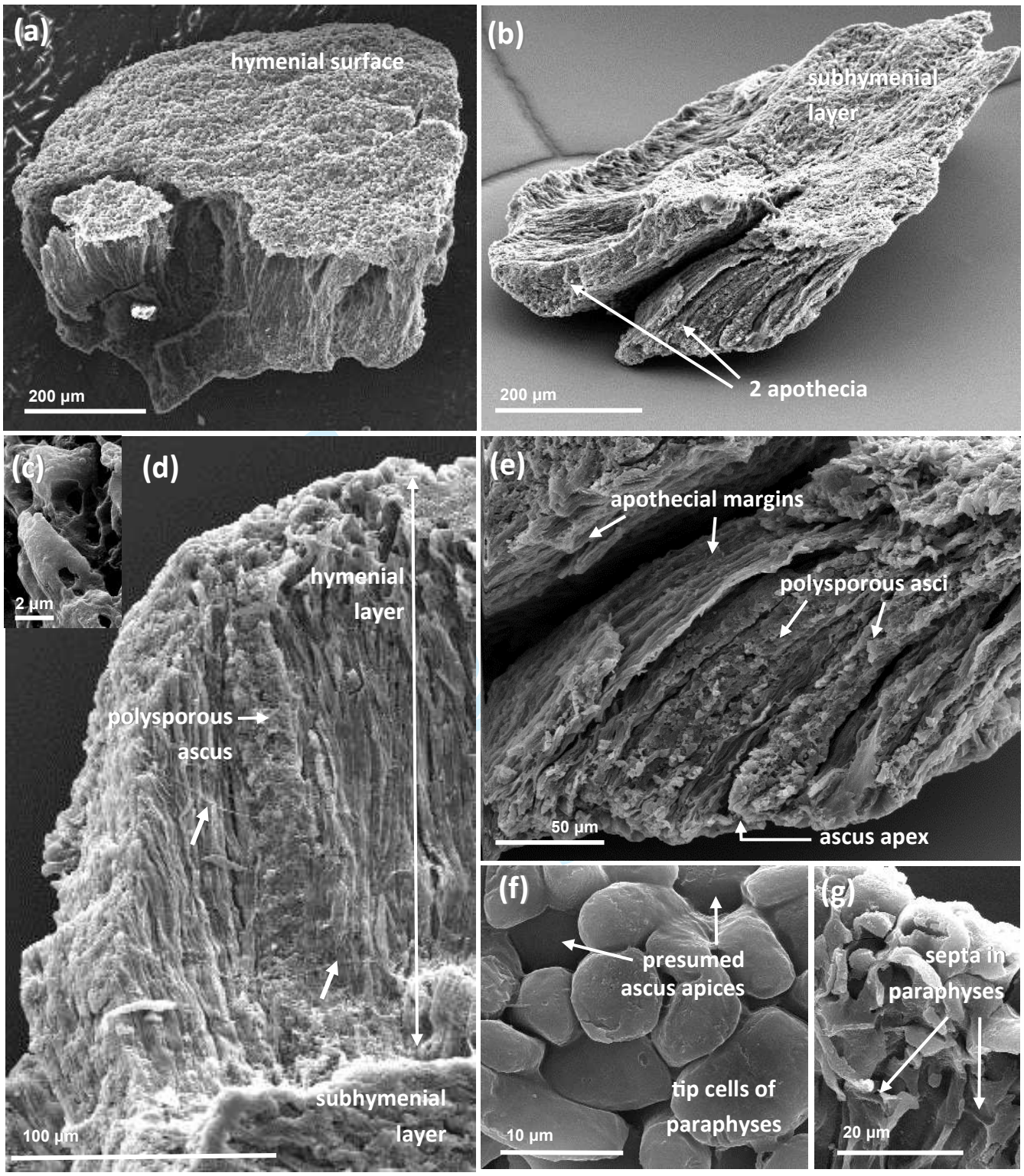
Figure 2 Slides with a petrographic thin section and a series of surface peels of *Prototaxites taiti,* as prepared by Prof. A.G. Lyon. (a) overview of slide 149 CT B with petrographic thin section (see details in Figs. 3-5) and serial peels in slides 149/B 2, 3, 5, 8 & 9. (b) detail of slide 149/B 8, the position of Figs. 1(c) and (d) being marked. (c) cross-section of a cord (or a very young axis?) with a somewhat denser peripheral zone. (d) slightly tangential section of the peripheral layer of a thick axis which, in the present study, is interpreted as the hymenial layer of an apothecium-bearing ascomycete. (e) confocal light micrograph of the hymenial layer of the type specimen of *P. taitii* (slide Nr. 2525 of the Kidston and Lang collection, see Fig. 1(a).


(a)
tip cells of paraphyses
paraphyses
polysporous asci
ascospores
100 µm
(b)
(c)
(d)
tip cells of paraphyses
(e)


(a)
30 µm
(b)
4 µm
(c)
actinobacterial filaments
fungal hyphae
100 µm
(d)
ascus tips
tip cells of paraphyses
100 µm
(e)
200 µm


(a)
500 µm
(b)
50 µm
(c)
epihymenium
hymenium
medullary spots
500 µm
(d)
20 µm
(e)
10 µm




(a)
hymenial surface
200 µm
(b)
subhymenial layer
2 apothecia
200 µm
(c)
2 µm
(d)
hymenial layer
polysporous ascus
subhymenial layer
100 µm
(e)
apothecial margins
polysporous asci
50 µm
ascus apex
(f)
presumed ascus apices
tip cells of paraphyses
10 µm
(g)
septa in paraphyses
20 µm

Taphrinomycotina ← ***Pezizomycotina*** →

Neolectomycetes *Geoglossomycetes* *Lecanoromycetes* (lichenized)